

PRELIMINARY AMENDMENT
NATIONAL STAGE ENTRY
OF PCT/GB03/01689

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application:

LISTING OF CLAIMS:

Claim 1. (Cancelled).

Claim 2. (Original) A method of culturing microorganisms which comprises bringing together culturing a microorganism and in a growth medium therefore, characterized in that said growth medium is or is prepared from a sterile nutrient composition derived from the biomass of a culture of bacteria including methanotrophic bacteria, wherein said culturing optionally with the addition occurs in the presence of further nutrients.

Claim 3. (Currently Amended) A use or The method as claimed in claim 1 or claim 2, wherein said nutrient composition comprises a hydrolysate, homogenizate or autolysate of said biomass, preferably an autolysate.

Claim 4. (Currently Amended) A use or The method as claimed in any one of claims 1 to 3 claim 2, wherein said growth medium further comprises glucose and/or nitrate and mineral salts (e.g. potassium, calcium, magnesium, sodium, molybdenum, iron, zinc, boron, cobalt, manganese and nickel compounds).

Claim 5. (Currently Amended) A use or The method as claimed in claim 4, wherein the glucose is present in said growth medium in a dry mass basis weight ratio of 5:1 to 1:5 (especially 2:1 to 1:2), relative to the biomass deriving component.

Claim 6. (Currently Amended) A use or The method as claimed in claim 4 or claim 5, wherein the nitrate and mineral

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salts are present in said growth medium in a weight ratio of 0.01:1 to 0.2:1 (~~especially 0.05:1 to 0.01:1~~), relative to the biomass deriving component.

Claim 7. (Currently Amended) ~~A use or~~The method as claimed in ~~any preceding~~ claim 2, wherein the culture of bacteria used to produce the biomass is at least 50%, preferably at least 60%, especially at least 70%, in particular at least 75%, e.g., 75 to 95%, more particularly 75 to 80%, by weight methanotrophic bacteria (relative to the total bacterial weight).

Claim 8. (Currently Amended) ~~A use or~~The method as claimed in ~~any preceding~~ claim 2, wherein said culture of bacteria comprises at least one species of methanotrophic bacteria and at least one species of heterotrophic bacteria.

Claim 9. (Currently Amended) ~~A use or~~The method as claimed in claim 8, wherein said culture comprises *Methylococcus capsulatus* (Bath) (strain NCIMB 11132), *Ralstonia* sp. DB3 (strain NCIMB 13287) and *Brevibacillus agri* DB5 (strain NCIMB 13289), optionally in combination with *Aneurinibacillus* sp. DB4 (strain NCIMB 13288).

Claim 10. (Currently Amended) ~~A use or~~The method as claimed in ~~any preceding~~ claim 2, wherein said culture of bacteria is produced by fermentation on hydrocarbon fractions or on natural gas, ~~preferably from fermentation on natural gas~~.

Claim 11. (Currently Amended) A microorganism growth substrate comprising a sterile nutrient composition derived from the biomass of a culture of bacteria including methanotrophic bacteria as defined in any one or claims 1 to 3 and 7 to 10, further comprising at least one sterile nutrient, and optionally containing a diluent.

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Claim 12. (Currently Amended) A The substrate as claimed in claim 11, wherein said sterile nutrient is selected from glucose, nitrate and mineral salts (e.g., potassium, calcium, magnesium, sodium, molybdenum, iron, zinc, boron, cobalt, manganese and nickel compounds), and combinations thereof.

Claim 13. (Currently Amended) A The substrate as claimed in claim 12, wherein the glucose is present in a dry mass basis weight ratio of 5:1 to 1:5 (especially 2:1 to 1:2), relative to the biomass deriving component.

Claim 14. (Currently Amended) A substrate as claimed in claim 12 ~~or claim 13~~, wherein the nitrate and mineral salts are present in a weight ratio of 0.01:1 to 0.2:1 (especially 0.05:1 to 0.01:1), relative to the biomass deriving component.

Claim 15. (New) The method as claimed in claim 3, wherein said nutrient composition comprises an autolysate of said biomass.

Claim 16. (New) The method as claimed in claim 4, wherein said mineral salts are selected from the group consisting of potassium, calcium, magnesium, sodium, molybdenum, iron, zinc, boron, cobalt, manganese and nickel compounds.

Claim 17. (New) The method as claimed in claim 5, wherein the glucose is present in said growth medium in a dry mass basis weight ratio of preferably 2:1 to 1:2, relative to biomass deriving component.

Claim 18. (New) The method as claimed in claim 6, wherein the nitrate and mineral salts are present in said growth medium in a weight ratio of preferably 0.05:1 to 0.01:1, relative to biomass deriving component.

Claim 19. (New) The method as claimed in claim 7, wherein the culture of bacteria used to produce the biomass is at

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least 60% by weight methanotrophic bacteria (relative to the total bacterial weight).

Claim 20. (New) The method as claimed in claim 19, wherein the culture of bacteria used to produce the biomass is at least 70% by weight methanotrophic bacteria (relative to the total bacterial weight).

Claim 21. (New) The method as claimed in claim 20, wherein the culture of bacteria used to produce the biomass is at least 75% by weight methanotrophic bacteria (relative to the total bacterial weight).

Claim 22. (New) The method as claimed in claim 21, wherein the culture of bacteria used to produce the biomass is 75% to 95% by weight methanotrophic bacteria (relative to the total bacterial weight).

Claim 23. (New) The method as claimed in claim 22, wherein the culture of bacteria used to produce the biomass is 75 to 80% by weight methanotrophic bacteria (relative to the total bacterial weight).

Claim 24. (New) The method as claimed in claim 10, wherein said culture of bacteria is produced by fermentation on natural gas.

Claim 25. (New) The substrate as claimed in claim 12, wherein said mineral salts are selected from the group consisting of potassium, calcium, magnesium, sodium, molybdenum, iron, zinc, boron, cobalt, manganese and nickel compounds.

Claim 26. (New) The substrate as claimed in claim 13, wherein the glucose is present in a dry mass basis weight ratio of preferably 2:1 to 1:2, relative to biomass deriving component.

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Claim 27. (New) The substrate as claimed in claim 14, wherein the nitrate and mineral salts are present in a weight ratio of preferably 0.05:1 to 0.01:1, relative to biomass deriving component.